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Comparison of *Campylobacter jejuni* Lipooligosaccharide Biosynthesis Loci from a Variety of Sources

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Campylobacter jejuni strains exhibit significant variation in the genetic content of the lipooligosaccharide (LOS) biosynthesis loci with concomitant differences in LOS structure. The _C. jejuni_ LOS loci have been grouped into six classes based on gene content and organization. Utilizing PCR amplifications of genes from these loci, we were able to classify a majority (80%) of the LOS biosynthesis loci from 123 strains of _C. jejuni_ that included 39 of the Penner serotype reference strains. We found that a particular LOS class was not always associated with a specific Penner serotype, and 14 of 16 Guillain-Barré syndrome-associated isolates tested in this study shared the same LOS class. The remaining isolates that could not be classified were often distinguishable from each other based on the results of gene-specific PCR and the lengths of their LOS biosynthesis loci as determined by long (XL) PCR. Sequence analysis of two of these unique XL PCR products demonstrated two new LOS classes. These results support the hypothesis that the LOS locus is a hot spot for genetic exchange and rearrangements. Analysis of the LOS biosynthesis genes by PCR assays can be used for typing _C. jejuni_ and offers the advantage of inferring potential LOS structures.

Campylobacter jejuni is the leading cause of acute bacterial gastroenteritis worldwide. Campylobacteriosis, in rare cases, is a likely antecedent to the development of peripheral neuropathies, Guillain-Barré syndrome (GBS), and Miller Fisher syndrome (29, 30). The lipooligosaccharides (LOS) of several *C. jejuni* strains have been shown to exhibit molecular mimicry with gangliosides concentrated in peripheral nerves, and it has been speculated that the peripheral neuropathies are related directly to autoimmune mechanisms following infection (20). However, not all strains of *C. jejuni* exhibit ganglioside mimicry, and it is estimated that fewer than 1 per 1,000 *Campylobacter* infections are followed by GBS (1, 18).

A cluster of genes involved in *C. jejuni* LOS biosynthesis was identified by analysis of the complete genome sequence of *C. jejuni* NCTC 11168; it extends from Cj1131c (*galE*) to Cj1151c (*rfaD*) (16, 17, 24). The results of both sequencing of LOS biosynthesis loci from other *C. jejuni* strains (5, 7, 10, 11) and microarray analysis (2, 14, 15, 25) indicated that the LOS biosynthesis locus is one of the more variable regions of the *C. jejuni* genome. Based on gene content and organization of the LOS biosynthesis loci, LOS classes A, B, and C have been described (7). Along with class-specific glycosyltransferases, these classes possess *neuBCA* genes that are required for sialic acid biosynthesis and a *cst* gene that encodes a sialic acid transferase (7, 10, 17). Thus, these three classes encode genes responsible for the production of sialylated LOS that are ganglioside mimics (7). Moreover, it was demonstrated that the LOS structures did not always correlate with a particular Penner serotype.

The Penner serotyping system relies on differences in *Campylobacter* heat-stable antigens, originally proposed to be LOS and/or lipopolysaccharide-type molecules (26, 27). Recently, capsular polysaccharides were shown to account for the Penner serotype specificity of several serotypes (12). To gain a greater understanding with regard to the diversity of the *C. jejuni* LOS loci and the potential relationship to Penner serotypes, we examined the composition of over 100 *C. jejuni* LOS biosynthesis loci. We were able to classify approximately 80% of the LOS loci and observed that over 60% of these loci belong to class A, B, or C. We also determined the genetic composition of two loci that were not classified.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used in this study are listed in Table 1. *C. jejuni* strains were cultured at 42°C under microaerophilic conditions (5% O_2, 10% CO_2, and 85% N_2) on *Brucella* agar supplemented with 0.025% (wt/vol) $FeSO_4 \cdot 7H_2O$, 0.025% (wt/vol) sodium metabisulfite (anhydrous), and 0.025% (wt/vol) sodium pyruvate (anhydrous).

Preparation of *C. jejuni* genomic DNA. *C. jejuni* cells were scraped from a plate and resuspended in 1.5 ml 10% (wt/vol) sucrose–50 mM Tris (pH 8.0), to which was added 250 μl of a 10-mg ml^{-1} lysozyme solution (in 250 mM Tris, pH 8.0), followed by 600 μl of 0.1 M EDTA. The suspension was incubated for 10 min on ice, and 300 μl of a 5% (wt/vol) sodium dodecyl sulfate solution was added and then briefly vortexed to clarify the solution. RNaseA (1 mg ml^{-1}) and proteinase K (10 mg ml^{-1}) were added, sequentially, and the lysates incubated for 30 min and 1 h at 37°C, respectively. Sodium acetate and ethanol were added, and DNA was removed by spooling onto a hooked Pasteur pipette. DNA was resuspended in Tris-EDTA (pH 8.0), extracted twice with phenol-chloroform (1:1, vol/vol) and once with chloroform, and concentrated by ethanol precipitation. Genomic DNA was also isolated from bacteria by using the DNeasy tissue kit (QIAGEN, Valencia, CA).

PCR. Conventional PCR reagents were supplied by Epicentre (Madison, WI). Each PCR consisted of 1× MasterAmp *Taq* PCR buffer, 1× MasterAmp *Taq* Enhancer, 2.5 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate, forward and reverse primers at 0.2 μM each, 0.2 U of MasterAmp *Taq* DNA polymerase (Epicentre) or *Taq* DNA polymerase (NEB, Beverly, MA), and approximately 50 ng of genomic DNA (final reaction volume, 25 μl). The LOS gene-specific

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TABLE 1. *Campylobacter jejuni* strains used in this study

Strain	Description[a]	Reference or source[b]
RM1862	NCTC 11168, UA580; Lior 4, Penner HS:2	Laboratory collection
RM1863	81116, UA501; Penner HS:6	Laboratory collection
RM1864	81-176; Penner HS:23,36	Laboratory collection
RM1045	MSCS7360 (ATCC 43429); Penner HS:1; human	P. Guerry
RM1046	CJC-25 (ATCC 43430); Penner HS:2; calf	P. Guerry
RM1047	TGH 9011 (ATCC 43431); Penner HS:3	P. Guerry
RM1048	ATCC 43432; Penner HS:4; human	P. Guerry
RM1049	Unknown	Laboratory collection
RM1050	MK198 (ATCC 43449); Penner HS:23; human	P. Guerry
RM1052	MK290 (ATCC 43456); Penner HS:36; human	P. Guerry
RM1155	CjT1 (#134); Lior 1; human	D. Woodward
RM1156	CjT2 (#195); Lior 2; human	D. Woodward
RM1158	CjT5 (#170); Lior 5; human	D. Woodward
RM1160	CjT7 (#35); Lior 7; human	D. Woodward
RM1163	CjT11 (#244); Lior 11; human	D. Woodward
RM1167	CjT28 (#1180); Lior 28; human	D. Woodward
RM1170	Penner HS:31; chicken	Laboratory collection
RM1188	Penner HS:2; chicken	Laboratory collection
RM1221	Penner HS:53; chicken	Laboratory collection; 19
RM1244	90A2737; human	S. Abbott
RM1245	96A5046; Penner HS:19,38; GBS	S. Abbott
RM1246	92A3120; Penner HS:7; human	S. Abbott
RM1247	96A11074; Penner HS:4,13,64,66; human	S. Abbott
RM1248	96A14504; Penner HS:4,13,50,64; human	S. Abbott
RM1285	Penner HS:19; chicken	Laboratory collection
RM1409	Penner HS:4,64; chicken	Laboratory collection
RM1413	Penner HS:10; chicken	Laboratory collection
RM1437	Penner HS:11; chicken	Laboratory collection
RM1443	Penner HS:38,63; chicken	Laboratory collection
RM1449	Penner HS:4,13,19,50,65; chicken	Laboratory collection
RM1464	Penner HS:4,13,16,19,50; chicken	Laboratory collection
RM1477	D445; Penner HS:19,38; human	Laboratory collection
RM1478	D226; Penner HS:2; human	Laboratory collection
RM1479	EDL18; Penner HS:17,23,36; human	Laboratory collection
RM1480	D1117; Penner HS:2; human	Laboratory collection
RM1501	Chicken	Laboratory collection
RM1507	LCDC #17384; Lior 16, Penner HS:10; human	W. Johnson
RM1508	LCDC #17385; Lior 11, Penner HS:53; human	W. Johnson
RM1510	LCDC #17402; Lior ND, Penner HS:19; GBS	W. Johnson
RM1511	LCDC #17403; Lior ND, Penner HS:19; GBS	W. Johnson
RM1516	CIP 702 (ATCC 33560); bovine	I. Wesley
RM1551	ATCC 43433; Penner HS:5; human	I. Wesley
RM1552	ATCC 43434; Penner HS:6; human	I. Wesley
RM1553	ATCC 43435; Penner HS:7; human	I. Wesley
RM1554	ATCC 43436; Penner HS:8; human	I. Wesley
RM1555	ATCC 43437; Penner HS:9; goat	I. Wesley
RM1556	ATCC 43438; Penner HS:10; human	I. Wesley
RM1844	D135; human	R Meinersmann
RM1845	D140; human	R Meinersmann
RM1847	D142; lamb	R Meinersmann
RM1849	D781; Lior 2,33, Penner HS:1,44; chicken	R Meinersmann
RM1850	D983; chicken	R Meinersmann
RM1851	D1038; chicken	R Meinersmann
RM1852	D1420; chicken	R Meinersmann
RM1853	D1730; human	R Meinersmann
RM1860	L18; Lior 18, Penner HS:55	R Meinersmann
RM1861	L19; Lior 19, Penner HS:42,15	R Meinersmann
RM1892	K21; chicken	L. Stanker
RM2227	72522; chicken	M. Englen
RM2769	Chicken	Laboratory collection
RM3145	HB93-13; Penner HS:19; GBS	I. Nachamkin (21)
RM3146	HB93-29; Penner HS:19; GBS	I. Nachamkin (21)
RM3147	INP7; Penner HS:19; GBS	I. Nachamkin (21)
RM3148	INP21; Penner HS:41; GBS	I. Nachamkin (21)
RM3149	INP59; Penner HS:41; GBS	I. Nachamkin (21)
RM3193	260.94; Penner HS:41; GBS	A. Lastovica (28)
RM3194	285.94; human	A. Lastovica
RM3196	233.94; Penner HS:41; GBS	A. Lastovica (13)

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TABLE 1—*Continued*

Strain	Description[a]	Reference or source[b]
RM3197	308.95; Penner HS:41; GBS	A. Lastovica (13)
RM3198	367.95; Penner HS:41; GBS	A. Lastovica (13)
RM3200	302.96; Penner HS:33; human	A. Lastovica
RM3201	378.96; Penner HS:41; human	A. Lastovica
RM3203	16.97; Penner HS:12; human	A. Lastovica
RM3204	20.97; Penner HS:12; human	A. Lastovica
RM3205	199.97; Penner HS:41; human	A. Lastovica
RM3206	231.97; Penner HS:41; human	A. Lastovica
RM3207	250.97; Penner HS:41; human	A. Lastovica
RM3208	1.98; Penner HS:21; human	A. Lastovica
RM3209	24.98; Penner HS:12; human	A. Lastovica
RM3210	242.98; Penner HS:8,17; human	A. Lastovica
RM3211	96.00; Penner HS:33; GBS	A. Lastovica
RM3264	17,387; Penner HS:2; GBS	D. Woodward
RM3265	98-1718; Penner HS:4,13,64; GBS	D. Woodward
RM3266	17,714; Penner HS:1; GBS	D. Woodward
RM3405	HS:1; Penner serotype reference strain	D. Woodward and C. Clark
RM3406	HS:2; Penner serotype reference strain	D. Woodward and C. Clark
RM3407	HS:3; Penner serotype reference strain	D. Woodward and C. Clark
RM3408	HS:4; Penner serotype reference strain	D. Woodward and C. Clark
RM3409	HS:5; Penner serotype reference strain	D. Woodward and C. Clark
RM3410	HS:6; Penner serotype reference strain	D. Woodward and C. Clark
RM3411	HS:7; Penner serotype reference strain	D. Woodward and C. Clark
RM3412	HS:8; Penner serotype reference strain	D. Woodward and C. Clark
RM3413	HS:9; Penner serotype reference strain	D. Woodward and C. Clark
RM3414	HS:10; Penner serotype reference strain	D. Woodward and C. Clark
RM3415	HS:11; Penner serotype reference strain	D. Woodward and C. Clark
RM3416	HS:13; Penner serotype reference strain	D. Woodward and C. Clark
RM3417	HS:16; Penner serotype reference strain	D. Woodward and C. Clark
RM3418	HS:17; Penner serotype reference strain	D. Woodward and C. Clark
RM3419	HS:18; Penner serotype reference strain	D. Woodward and C. Clark
RM3420	HS:19; Penner serotype reference strain	D. Woodward and C. Clark
RM3421	HS:22; Penner serotype reference strain	D. Woodward and C. Clark
RM3422	HS:23; Penner serotype reference strain	D. Woodward and C. Clark
RM3423	HS:27; Penner serotype reference strain	D. Woodward and C. Clark
RM3424	HS:29; Penner serotype reference strain	D. Woodward and C. Clark
RM3425	HS:32; Penner serotype reference strain	D. Woodward and C. Clark
RM3426	HS:35; Penner serotype reference strain	D. Woodward and C. Clark
RM3427	HS:36; Penner serotype reference strain	D. Woodward and C. Clark
RM3428	HS:37; Penner serotype reference strain	D. Woodward and C. Clark
RM3429	HS:38; Penner serotype reference strain	D. Woodward and C. Clark
RM3430	HS:41; Penner serotype reference strain	D. Woodward and C. Clark
RM1503	HS:43; Penner serotype reference strain	W. Johnson
RM3431	HS:44; Penner serotype reference strain	D. Woodward and C. Clark
RM3432	HS:45; Penner serotype reference strain	D. Woodward and C. Clark
RM3433	HS:50; Penner serotype reference strain	D. Woodward and C. Clark
RM3434	HS:52; Penner serotype reference strain	D. Woodward and C. Clark
RM3435	HS:53; Penner serotype reference strain	D. Woodward and C. Clark
RM3436	HS:57; Penner serotype reference strain	D. Woodward and C. Clark
RM3437	HS:56; Penner serotype reference strain	D. Woodward and C. Clark
RM3438	HS:60; Penner serotype reference strain	D. Woodward and C. Clark
RM3439	HS:62; Penner serotype reference strain	D. Woodward and C. Clark
RM3440	HS:63; Penner serotype reference strain	D. Woodward and C. Clark
RM3441	HS:64; Penner serotype reference strain	D. Woodward and C. Clark
RM3442	HS:65; Penner serotype reference strain	D. Woodward and C. Clark

[a] Given as strain name; Penner type; clinical (GBS) or environmental origin.

[b] Affiliations for providers of strains are as follows: P. Guerry, Naval Medical Research Institute (NMRI), Bethesda, Md.; D. Woodward and C. Clark, National Microbiology Laboratory, Winnipeg, Canada; W. Johnson, Laboratory for Enteric Pathogens, Winnipeg, Canada; S. Abbott, California State Public Health Lab, Berkeley, Calif.; R. Meinersmann, USDA, Agricultural Research Service (ARS), Athens, Ga.; I. Wesley, USDA, ARS, NADC, Ames, Iowa; L. Stanker, USDA, ARS, Albany, Calif.; M. Nicholson, Centers for Disease Control and Prevention, Atlanta, Ga.; M. Englen, USDA, ARS, Athens, Ga.; I. Nachamkin, University of Pennsylvania, Philadelphia, Pa.; A. Lastovica, University of Cape Town, Cape Town, South Africa.

primers are described in Table 2. Amplification conditions for all LOS gene-specific products occurred under the following parameters: 30 cycles of 25 s at 94°C, 25 s at 52°C, and 1 min at 72°C and a final extension at 72°C for 5 min. Long PCR (XL PCR) reagents were supplied by Epicentre (Madison, WI). Each XL PCR consisted of 1× MasterAmp Extra-Long PreMix 5, LOSXL primers (Table 2) at 0.2 μM each, 2.5 U of MasterAmp Extra-Long DNA polymerase, and 250 ng of genomic DNA (final reaction volume, 50 μl). The cycling conditions for XL PCR products were 25 cycles of 30 s at 94°C, 45 s at 52°C, and 15 min at 68°C and a final extension at 68°C for 15 min. Thermal cycling was performed with a Tetrad thermal cycler (MJ Research, Waltham, MA). All PCR products were analyzed by agarose gel electrophoresis. Positive samples were identified based on the presence of bands of anticipated sizes. Primers were purchased from either Operon Technologies (Alameda, CA) or Sigma-Genosys (The Woodlands, TX).

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TABLE 2. Primers used in this study

ORF	Primer 1	Primer 2
orf2	5′ CAAATCTGTTTCCCTCAATACACTCA	5′ TTTAATCTTACGCTTTCGTTTTCTAC
orf3bf	5′ ACAAAGAAGTGAATTATCAAATGGGAGC	5′ TTGCCCAAAGGCTTGAGTAGTGCTG
orf3ac2	5′ AAGATAATATCATCGCACTTAGTCGTAAA	5′ ACAAACTCACTATCTTCCCTACCCC
orf3e	5′ AATAGATAGCGGAAGCACAGATGA	5′ CTATGATAAAACCTCTTTTGCCTTCTA
orf4ab	5′ GAATCAAACTTATACTAACTTAGAAATC	5′ CATTTGCTTTTGTAATCTTCTT
orf4c	5′ AAAATGGTGGTTTAAGTAGTGCTAG	5′ GTTTGGATAAAAGGTTTAATTATAGGT
orf5/10c	5′ AGTATGTTACCTGCCATACAAAGAGG	5′ GGTATGCAATACAACCTTATCACTAG
orf5ab1	5′ TTCATCC CTTTTAGAAAAATTAGAC	5′ AAGCAAGCAATCTCCTGGTTC
orf5ab2	5′ TTCATCC CTTTTAGAAAAATTAGAC	5′ TGCTAAACAATCTCCAGGAGC
orf5bII	5′ CTGTGATGATGGGAGTGAAGAGC	5′ GGTAATCGTTTCGGCGGTATT
orf6ab1	5′ CAAGGGCAATAGAAAGCTGTATCA	5′ ACAAGCACTTCATTCTTAGTATTACAAAT
orf6ab2	5′ TCATCTTGCCAACTTATAATGTGGA	5′ TCTAGCGATATTAAACCAACAGCCT
orf6c	5′ GTAGTAGATGATTGTGGTAATGATAAA	5′ ATAGAATTGCTATTTACATGCTGG
orf7ab	5′ ACTACACTTTAAAACATTTAATCC AAAATCA	5′ CCATAAGCCTCACTAGAAGGTATGAGTATA
orf7c	5′ TTGAAGATAGATATTTTGTGGGTAAA	5′ CTTTAAGTAGTGTTTTATGTCACTTGG
orf8ab	5′ ATTATAGCCATTTGCTCACTTTG	5′ AAAGCACCCTTAGTCGTACCTG
orf8c	5′ CCTTTGATAATCC CTGAAATAGGT	5′ TCCTTTGCACTTATACCACCTT
orf9ab	5′ AGCAGCAGCTATTGTTGGAGCAT	5′ TTCATTGCCAAGTCTTCCATTT
orf9c	5′ TTTTGTTAGCGGAACTAGAGCTG	5′ TTATTTCGGTTGTAATTGGATGA
orf10ab	5′ TGCTCGTGGTGGCTCAAAGGGTA	5′ CCTGCTAAATCGCCATAATCATTACAAACAA
orf11ab	5′ GAAATGGGTTCATTTTCTTTTAGCG	5′ TCTTCTAGTAGGTCAAGATATTGGTTTA
orf12	5′ GCCACAACTTTCTATCATAATCC CGC	5′ CGCCGTAACTCAAACGCTCATCTATT
orf14c	5′ GCTAGAACACCCTAAAGTGACTAA	5′ TGGCACTAAATTGTAATAAATGGC
orf15c	5′ AAACCGTAGGTGTAGTAATCC CC	5′ CATGATAATTTTCTACAAATCGCACT
orf16c	5′ AGGTATTGGTTTAATGGAGCTTTT	5′ GGTCCTATAACACCCCACGAGAT
orf16df	5′ GAAATTCTTACGATTAAATCTTGGC	5′ GTGAAGTTGTGATTCTAGCTTTGC
orf17d	5′ TTGAACAACCTGCTTATGAGCTTTAT	5′ TTTCTTTAGTGAATCTTCCCACGC
orf18df	5′ GCAGCAAGAAATAATGGTGTTAAAC	5′ AAATAATCATCC AAACATTCCTGAA
orf19df	5′ AAAATTTCCGTCATAATCC CAATCT	5′ TATCAGGTAAATCTTGAATGATAAAGTCA
orf20df	5′ GTCTTTTAAGAGCTAGATATGAAGGAG	5′ ATTAATGCATCTTCTGCCATAATTA
orf21e	5′ TATTAAATAGGAGTGCAACAATGAAAGG	5′ GCACAAACGAACTAGCTTCTATAAGACT
orf22e	5′ TATTTTAGTTACTGGCGGAGCTGG	5′ GAATAAGGGGAATTTGGAGCATAA
orf25e	5′ TATATGGCAATTTTGCGTAGTTTTA	5′ GCAATAATAAGTTGTATTGGCTGCA
orf26e	5′ ATATTGCCGTTAATTCATTACAGTT	5′ TTTGAGCGATAATTTTAAATCC ATC
orf27e	5′ GTAGATGATTGTTCAAATGATAATAGCACA	5′ GTTTTCAGATTCTAAGGCCATTATTCC
orf28e	5′ CAAATATACTATTGATTCTGGATAAGTGA	5′ TAACTATAAAATCATAGGAGGCATAT
orf29e	5′ ATGGGCTTGATGCTTTAAGATTGAT	5′ TTGGCGATATGGGTAATGACAAAA
orf30e	5′ TTTAGTTATGGTGTAAGTGGGCATTG	5′ TACCATATTTTCAAGTCTTTCATTCGC
orf31e	5′ TTTATACCAGTAAAATACCCTTATGAAG	5′ TTTTTCTTTAGTTACCATATCAGGTG
orf32e	5′ CAAGCTGTTGATGGAAAAGAGTTG	5′ TGGGCTCCAAATATCTCATCAGTAG
orf33e	5′ GATTTATTAAGAGTTTCTTTGCTCAA	5′ ATTTTATCTATCATGGAATGTTTTCTAA
orf34e	5′ ATGATCGATAATGAAATTACGATAGTAAC	5′ CGTCATCAATTATTCCAAATGAGGTA
LOSXL	5′ AAGCGTCCTATTATCTTCACAACTGCACACTATGG	5′ ATGCCACAACTTTCTATCATAATCC CGCTT

DNA sequencing. Cycle sequencing reactions were performed using the ABI PRISM BigDye terminator cycle sequencing kit (version 3.0). All extension products were purified on DyeEx spin columns (QIAGEN, Valencia, CA) or Centri-Sep spin columns (Princeton Separations, Princeton, NJ). DNA sequencing was performed using an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) with the POP-6 polymer and ABI PRISM Genetic Analyzer Data Collection and ABI PRISM Genetic Analyzer Sequencing Analysis software. Sequencing oligonucleotide primers were purchased from Operon Technologies (Alameda, CA).

Nucleotide sequence accession numbers. Nucleotide sequences have been deposited with GenBank and assigned accession numbers as follows: RM1170 LOS loci, AY434498; RM1555 (ATCC 42437) LOS loci, AY436358; RM1047 (ATCC 43431), AY800272.

RESULTS AND DISCUSSION

Specificity of LOS biosynthesis locus class-specific PCR primers. Based on gene content and organization of the LOS biosynthesis loci from 15 different strains of *C. jejuni* (3, 5, 7, 8, 10, 11, 22, 23), we grouped the loci into six major LOS classes: A, B, C, D, E, and F (Fig. 1). Classes A, B, and C, involved in sialylated LOS synthesis, were previously described (7). The sequences for classes D, E, and F were obtained from GenBank (3, 8, 22, 23) Each locus possesses a number of novel putative glycosyltransferase genes and genes with unknown function but lacks the *neuBCA* and *cst* genes and likely directs the synthesis of nonsialylated LOS structures. We evaluated 42 primer pairs (Table 2) specific to the genes within the six classes designated in Fig. 1 by PCR amplification of genomic DNA from strains representing each defined LOS class (Table 3). All *C. jejuni* LOS classes were amplified with the *waaM* (orf2) and *waaV* (orf12) primer sets. Strains RM1048, RM1556, RM1050, and RM1052 (classes A and B) share many amplification products, including *cstII* (orf7ab), *neuB1*-ab (orf8ab), *neuC1*-ab (orf9ab), *neuA1*-ab (orf10ab), and orf11ab. The *cgtA2* (orf5bII) product, however, is specific for class B and can be used to distinguish class A and class B. Previous work (7) demonstrated sequence divergence of a region spanning the *cgtA* (orf5abI) and *cgtB* (orf6ab) genes (~1,200 bp), creating two sets of alternative alleles: orf5abI1 and orf6ab1 or orf5abI2 and orf6ab2. These alternative alleles are present in both class A

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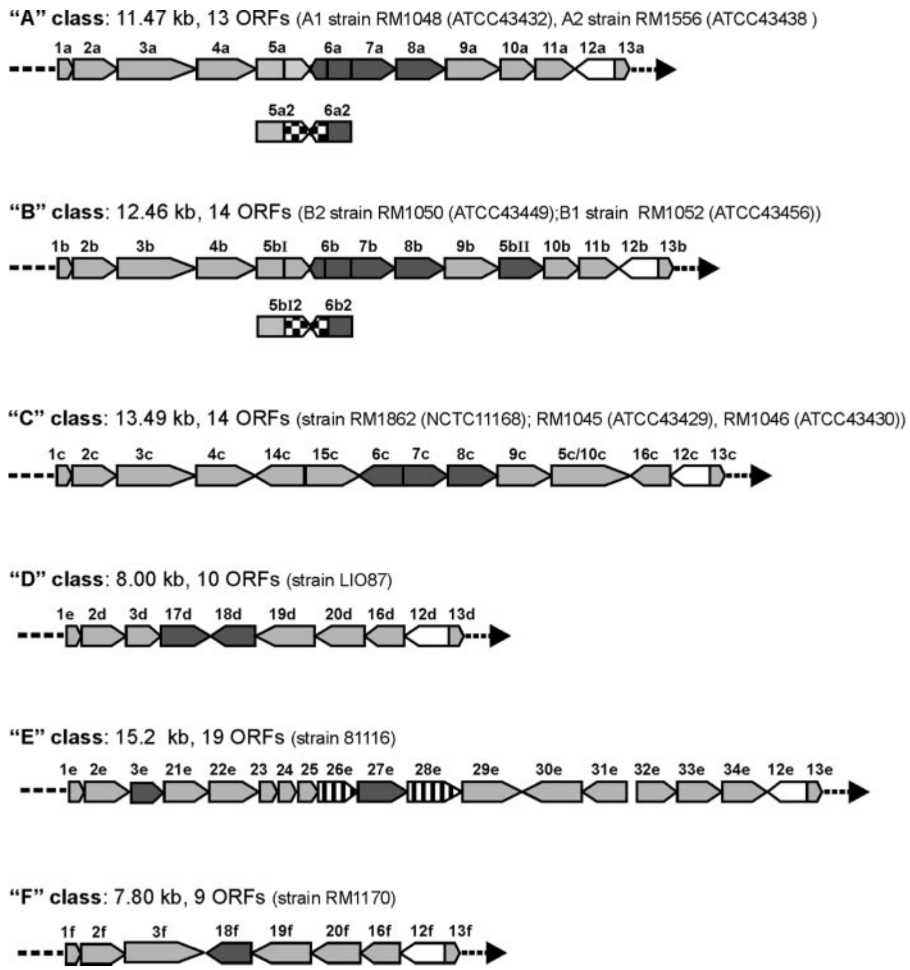

FIG. 1. Genetic organization of the six LOS biosynthesis locus classes of the different *C. jejuni* strains. The 12 ORFs used to survey putative LOS classes are shown in dark gray. The alternate alleles for orf5abI and orf6ab are shown as checkerboard regions. The *waaV* gene (orf12) is shown in white. The GenBank accession numbers are AF215659 for ATCC 43432 (strain RM1048), AF400048 for ATCC 43438 (RM1556), AF401529 for ATCC 43449 (RM1050), AF401528 for ATCC 43456 (RM1052), AL139077 for NCTC 11168 (RM1862), AY044156 for ATCC 43429 (RM1045), AF400047 for ATCC 43430 (RM1046), AF400669 for LIO87, AF343914 and AJ131360 for 81116 (RM1863), and AY434498 for RM1170.

and class B. We designed allele-specific primers for these genes, such that we amplified orf5abI1 and orf6ab1 from strains RM1048 (ATCC 43432) (class A1) and RM1052 (ATCC 43456) (class B1) but not orf5abI2 or orf6ab2. This allowed us to designate the alleles for class A (A1 and A2) and class B (B1 and B2).

The products for Cj1137c (orf14c), Cj1138 (orf15c), Cj1139c (orf6c), *cstIII* (orf7c), *neuB1*-c (orf8c), *neuC1*-c (orf9c), *cgtAneuA1*-c (orf5c/10c), and orf16c were amplified specifically from the class C loci. Classes D and F share many amplification products, including orf18d, orf19df, orf20df, and orf16df. The orf17d product, specific for class D, can distinguish classes D and F. Only class E *C. jejuni* strains produced PCR products for the orf21e, 22e, 25e, 26e, 27e, 28e, 29e, 30e, 31e, 32e, 33e, and 34e genes. Surprisingly, strain 81116 (class E) produced amplification products with *neuB1*-ab (orf8ab) and *neuC1*-ab (orf9ab). The identities of these products were confirmed by sequencing and suggest the presence of these genes elsewhere in the genome.

LOS biosynthesis locus diversity of the Penner serotype reference strains. From the class-specific primer pairs, we identified 12 primer pairs representing genes from the six classes (orf6ab1, orf6ab2, orf7ab, orf8ab, orf5bII, orf6c, orf7c, orf8c, orf17d, orf18df, orf3e, and orf27e) that would allow putative class determination and distinguish between the two alleles of classes A and B. As a positive control for the presence of a *C. jejuni* LOS locus, we amplified the orf12 (*waaV*) product. Thirty-nine Penner serotype reference strains were examined. Twenty-five of the reference strains (HS:1 to HS:8, HS:10, HS:11, HS:13, HS:16, HS:18, HS:19, HS:23, HS:27, HS:29, HS:32, HS:35, HS:36, HS:41, HS:44, HS:45, HS:62, and HS:64) showed PCR-amplification patterns consistent with possessing class A1, A2, B1, B2, C, D, E, or F (Table 4). For these strains, we attempted to verify the LOS classes by additional amplifications of genes within the presumed class. Most strains had patterns consistent with their LOS class designation; however, we identified a number of strains with patterns that diverged from the expected class patterns. Most presumptive class E strains—RM3407 (HS:3), RM3411 (HS:7), RM3428 (HS:37), RM3429 (HS:38), and RM3432 (HS:45)—failed to produce two class E primer amplicons, orf26e and orf28e, while RM3423 (HS:27) failed to produce the orf28e amplicon. The

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TABLE 3. Validation of primer pairs for LOS biosynthesis ORFs

Strain	Penner type (HS)	LOS locus class	Amplification with primers specific to the following ORF: orf2: *waaM*	orf12: *waaV*	orf3bf	orf4ab	orf5ab11: *cgtA*-1	orf5ab12: *cgtA*-2	orf6ab1: *cgtB*-1	orf6ab2: *cgtB*-2	orf7ab: *cstII*	orf8ab: *neuB*-a	orf9ab: *neuC*-a	orf10ab: *neuA*	orf11ab	orf5bII: cgtA2
RM1048 (ATCC 43432)	4	A1	+	+	−	+	+	−	+	−	+	+	+	+	+	−
RM1556 (ATCC 43438)	10	A2	+	+	−	+	−	+	−	+	+	+	+	+	+	−
RM1052 (ATCC 43456)	36	B1	+	+	+	+	+	−	+	−	+	+	+	+	+	+
RM1050 (ATCC 43449)	23	B2	+	+	+	+	−	+	−	+	+	+	+	+	+	+
RM1045 (ATCC 43429)	1	C	+	+	−	−	−	−	−	−	−	−	−	−	−	−
RM1046 (ATCC 43430)	2	C	+	+	−	−	−	−	−	−	−	−	−	−	−	−
RM1862 (NCTC 11168)	2	C	+	+	−	−	−	−	−	−	−	−	−	−	−	−
LIO87	53	D	+	+	−	−	−	−	−	−	−	−	−	−	−	−
RM1863 (81116)	6	E	+	+	−	−	−	−	−	−	−	+	+	−	−	−
RM1170	31	F	+	+	+	−	−	−	−	−	−	−	−	−	−	−

Strain	orf3C: Cj1135	orf4c: Cj1136	orf14c: Cj1137c	orf15c: Cj1138	orf6c: Cj1139c	orf7c: *cstIII*	orf8c: *neuB* 1-c	orf9c: *neuC* 1-c	orf5c/10c: Cj1143	orf16c: Cj1144
RM1048 (ATCC 43432)	+	−	−	−	−	−	−	−	−	−
RM1556 (ATCC 43438)	+	−	−	−	−	−	−	−	−	−
RM1052 (ATCC 43456)	−	−	−	−	−	−	−	−	−	−
RM1050 (ATCC 43449)	−	−	−	−	−	−	−	−	−	−
RM1045 (ATCC 43429)	+	+	+	+	+	+	+	+	+	+
RM1046 (ATCC 43430)	+	+	+	+	+	+	+	+	+	+
RM1862 (NCTC 11168)	+	+	+	+	+	+	+	+	+	+
LIO87	−	−	−	−	−	−	−	−	−	−
RM1863 (81116)	−	−	−	−	−	−	−	−	−	−
RM1170	−	−	−	−	−	−	−	−	−	−

Strain	orf17d	orf18df	orf19df	orf20df	orf16df	orf3e	orf22e	orf23e	orf25e	orf26e	orf27f	orf28f	orf29f	orf30f	orf31f	orf32f	orf33f	orf34f
RM1048 (ATCC 43432)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
RM1556 (ATCC 43438)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
RM1052 (ATCC 43456)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
RM1050 (ATCC 43449)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
RM1045 (ATCC 43429)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
RM1046 (ATCC 43430)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
RM1862 (NCTC 11168)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
LIO87	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
RM1863 (81116)	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
RM1170	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−

sizes of the XL PCR products for the LOS loci of these strains (extending from orf2 [*waaM*] to orf12 [*waaV*]) were approximately 1 kb smaller than those of the class E strains. Similarly, the presumptive class D strain RM3418 (HS:17) and the presumptive class F strain RM3437 (HS:58) failed to produce the orf19df product and had XL PCR products that were slightly larger than those of the class D and class F strains (data not shown).

The remaining 15 Penner serotype reference strains (HS:9, HS:17, HS:22, HS:32, HS:37, HS:38, HS:43, HS:50, HS:52, HS:53, HS:57, HS:58, HS:60, HS:63, and HS:65) failed to produce PCR amplification patterns consistent with the six classes by use of the 12 primers. Some of these unclassified LOS loci possessed LOS genes from known classes. As with RM3407, these loci may represent a new class that is derived from a known class. The LOS loci of these strains are likely novel, or they possess extensive sequence divergence that prevents PCR amplification. Interestingly, strains RM3434 (HS:52) and RM3442 (HS:65) appeared to exhibit multiple LOS classes: B2 and C for RM3434 and A2 and F for RM3442. The XL PCR products for RM3434 and RM3442 are similar in size to the C and A2 XL PCR products, respectively. This suggests that these LOS loci are not likely a combination of the two classes. DNA sequencing will be required to determine the exact LOS class for each of these isolates and to address where the genes from the other LOS class are.

LOS classes of isolates from clinical and environmental sources. To investigate any associations between LOS classes and isolate sources, we screened 70 clinical and environmental isolates with the 12 primer pairs. We surveyed 16 GBS-associated isolates and found that 14 were positive for class A1 products, whereas the other 2 isolates were positive for ganglioside-associated classes A2 and C (Table 5). This is consistent with a study by Nachamkin et al. (21), who previously demonstrated a strong association of *cst-II* (orf7a,b), *cgtA* (orf5), and *cgtB* (orf6) with GBS isolates. It should be noted that most of the GBS-associated strains in this study (12 of 16 GBS strains) were HS:19 or HS:41, which have been shown to be genetically clonal (13, 28). This overrepresentation certainly increases the occurrence of the class A1 locus in this study. Examining additional non-HS:19 and non-HS:41 GBS-associated strains would provide a better indication of the relationship between LOS class and this syndrome. Indeed, Godschalk et al. recently examined 17 GBS-associated isolates and found that 53% possessed the class A locus (9). Interestingly, they also found that 18% of the GBS-associated strains possessed LOS loci with no known sialic acid transferase gene. These strains may possess a unique sialic acid transferase that allows the synthesis of ganglioside mimics. It is also possible that these strains coinfected with other *C. jejuni* strains capable of synthesizing a ganglioside mimic and are merely the strains recovered by culturing.

For other clinical isolates and for environmental isolates (Table 6), we observed strains with putative LOS locus classes A, B, C, D, E, and F, and we were also able to reclassify the LOS locus for several the putative class E isolates to class H (described below) by using the orf26e primer pair. As with the Penner serotyping reference strains, the LOS locus class of several isolates could not be determined by the PCR screen. The most striking finding was that 64% (35 of 55) of the

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TABLE 4. Survey of the LOS classes for Penner serotype reference strains

Strain	Penner serotype	Putative LOS class[a]	Amplification with primers specific to the following ORF[b]:															
			waaV	orf6ab1	orf6ab2	orf7ab	orf8ab	orf11	orf6c	orf7c	orf8c	orf17d	orf18d	orf3e	orf27e	**orf19d**	**orf16d**	**orf26e**
RM3408	4	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM3416	13	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM3417	16	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM3420	19	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM3430	41	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM3414	10	A2	+	−	+	+	+	−	−	−	−	−	−	−	−			
RM3424	29	B1	+	+	−	+	+	+	−	−	−	−	−	−	−			
RM3427	36	B1	+	+	−	+	+	+	−	−	−	−	−	−	−			
RM3409	5	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM3422	23	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM3426	35	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM3439	62	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM3405	1	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM3406	2	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM3412	8	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM3431	44	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM3418	17	D to Unk	+	−	−	−	−	−	−	−	−	+	+	−	−	−	+	
RM3410	6	E	+	−	−	−	+	−	−	−	−	−	−	+	+			+
RM3423	27	E	+	−	−	−	−	−	−	−	−	−	−	+	+			+
RM3407	3	E to H[c]	+	−	−	−	−	−	−	−	−	−	−	+	+			−
RM3411	7	E to H[c]	+	−	−	−	+	−	−	−	−	−	−	+	+			−
RM3432	45	E to H[c]	+	−	−	−	+	−	−	−	−	−	−	+	+			−
RM3415	11	F	+	−	−	−	−	−	−	−	−	−	+	−	−	+	+	
RM3419	18	F	+	−	−	−	−	−	−	−	−	−	+	−	−	+	+	
RM3441	64	F	+	−	−	−	+	−	−	−	−	−	+	−	−	+	+	
RM3437	58	F to Unk	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	
RM3413	9	Unk	+	−	−	−	−	−	−	−	−	−	−	+	−			
RM3421	22	Unk	+	+	+	+	+	+	−	−	−	−	−	−	−			
RM3425	32	Unk	+	+	−	−	+	−	−	−	−	+	−	−	−			
RM3428	37	Unk	+	−	−	+	+	−	−	−	−	−	−	+	+			
RM3429	38	Unk	+	−	−	+	+	−	−	−	−	−	−	+	+			
RM1503	43	Unk	+	−	−	−	+	−	−	−	−	+	−	−	−			
RM3433	50	Unk	+	−	−	+	+	−	−	−	−	−	−	−	−			
RM3434	52	Unk	+	−	+	+	+	+	+	+	+	−	−	−	−			
RM3435	53	Unk	+	−	−	−	−	−	−	−	−	−	−	+	−			
RM3436	57	Unk	+	−	−	−	−	−	−	−	−	−	−	−	−			
RM3438	60	Unk	+	−	−	−	−	−	−	−	−	−	−	−	+			
RM3440	63	Unk	+	−	−	−	−	−	−	−	−	+	−	−	−			
RM3442	65	Unk	+	−	+	+	+	−	−	−	−	−	+	+	−			

[a] Unk, unknown LOS class. D to Unk, the class designation was changed from D to unknown.
[b] Boldfaced ORFs were used to verify LOS class D, E, or F.
[c] Absence of orf26e changed the class designation from E to H.

TABLE 5. Survey of LOS classes for *C. jejuni* isolates associated with GBS

Strain	Penner serotype	Putative LOS class	Amplification with primers specific for the following ORF:												
			waaV	orf6ab1	orf6ab2	orf7ab	orf8ab	orf5bII	orf6c	orf7c	orf8c	orf17d	orf18df	orf3e	orf27e
RM3264	2	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM1510	19	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM1511	19	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3145	19	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3146	19	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3147	19	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3211	33	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3148	41	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3149	41	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3193	41	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3196	41	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3198	41	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM1245	19, 38	A1	+	+	−	+	+	−	−	−	−	−	−	−	−
RM3265	4, 13, 64	A2	+	−	+	+	+	−	−	−	−	−	−	−	−
RM3266	1	C	+	−	−	−	−	−	+	+	+	−	−	−	−

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TABLE 6. Survey of LOS classes for *C. jejuni* clinical and environmental isolates

Strain	Penner serotype[a]	Source	Putative LOS class[b]	Amplification with primers specific for the following ORF[c]:															
				waaV	orf6ab1	orfab2	orb7ab	orf8ab	orf5bII	orf6c	orf7c	orf8c	orf17d	orf18df	orf3e	orf27e	**orf19df**	**orf16df**	**orf26e**
RM1507	10	Human	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM1285	19	Chicken	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM3201	41	Human	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM3205	41	Human	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM3206	41	Human	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM3207	41	Human	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM1477	19, 38	Human	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM1464	4, 13, 16, 19, 50	Chicken	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM1449	4, 13, 19, 50, 65	Chicken	A1	+	+	−	+	+	−	−	−	−	−	−	−	−			
RM1413	10	Turkey	A2	+	−	+	+	+	−	−	−	−	−	−	−	−			
RM1160	ND	Human	A2	+	−	+	+	+	−	−	−	−	−	−	−	−			
RM1551	5	Human	B1	+	+	−	+	+	+	−	−	−	−	−	−	−			
RM1864 (81–176)	23, 36	Human	B1	+	+	−	+	+	+	−	−	−	−	−	−	−			
RM1248	4, 13, 50, 64	Human	B1	+	+	−	+	+	+	−	−	−	−	−	−	−			
RM1158	ND	Human	B1	+	+	−	+	+	+	−	−	−	−	−	−	−			
RM1852	ND	Chicken	B1	+	+	−	+	+	+	−	−	−	−	−	−	−			
RM3194	ND	Human	B1	+	+	−	+	+	+	−	−	−	−	−	−	−			
RM1188	2	Chicken	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM1516	23, 36	Cow	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM1247	4, 13, 64, 66	Human	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM1409	4, 84	Turkey	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM1155	ND	Human	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM1156	ND	Human	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM1244	ND	Human	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM1501	ND	Chicken	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM1851	ND	Chicken	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM2769	ND	Chicken	B2	+	−	+	+	+	+	−	−	−	−	−	−	−			
RM1478	2	Human	C	+	−	−	−	+	−	+	+	+	−	−	−	−			
RM1480	2	Human	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM1554	8	Human	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM1437	11	Turkey	C	+	−	−	−	−	−	+	+	+	−	+	−	−			
RM1844	ND	Cow	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM1845	ND	Human	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM1847	ND	Lamb	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM1849	ND	Chicken	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM1853	ND	Human	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM1892	ND	Chicken	C	+	−	−	−	−	−	+	+	+	−	−	−	−			
RM1861	42, 15	Human	D	+	−	−	−	−	−	−	−	−	+	+	−	−	+	+	
RM1850	ND	Chicken	D to Unk	+	−	−	−	−	−	−	−	−	+	+	−	−	+	−	
RM1552	6	Human	E	+	−	−	−	+	−	−	−	−	−	−	+	+			+
RM1860	55	Human	E	+	−	−	−	−	−	−	−	−	−	−	+	+			+
RM1047 (ATCC 43431)	3	Human	E to H[d]	+	−	−	−	+	−	−	−	−	−	−	+	+			−
RM1246	7	Human	E to H[d]	+	−	−	−	+	−	−	−	−	−	−	+	+			−
RM1553	7	Human	E to H[d]	+	−	−	−	+	−	−	−	−	−	−	+	+			−
RM3203	12	Human	E to H[d]	+	−	−	−	−	−	−	−	−	−	−	+	+			−
RM3204	12	Human	E to H[d]	+	−	−	−	−	−	−	−	−	−	−	+	+			−
RM3209	12	Human	E to H[d]	+	−	−	−	−	−	−	−	−	−	−	+	+			−
RM3208	21	Human	E to H[d]	+	−	−	−	−	−	−	−	−	−	−	+	+			−
RM1167	37	Human	E to H[d]	+	−	−	−	−	−	−	−	−	−	−	+	+			−
RM1221	53	Chicken	F	+	−	−	−	−	−	−	−	−	−	+	−	−	+	+	
RM1163	53	Human	F to Unk	+	−	−	−	−	−	−	−	−	−	+	−	−	+	−	
RM1508	53	Human	F to Unk	+	−	−	−	−	−	−	−	−	−	+	−	−	+	−	
RM2227	15	Chicken	F to Unk	+	−	−	−	−	−	−	−	−	−	+	−	−	+	−	
RM3200	33	Human	Unk	+	+	+	+	+	+	−	−	−	−	−	−	−			
RM1479	17, 23, 36	Human	Unk	+	−	−	+	+	+	−	−	−	−	−	−	−			
RM1443	38, 63	Turkey	Unk	+	−	−	−	−	−	−	−	−	−	−	+	−			
RM1555	9	Goat	Unk to G[e]	+	−	−	−	−	−	−	−	−	−	−	+	−			

[a] ND, not determined.
[b] Unk, unknown LOS class. D to Unk, the class designation was changed from D to unknown.
[c] Boldfaced ORFs were used to verify LOS class D, E, or F.
[d] Absence of orf26e changed the class designation from E to H.
[e] The class designation was changed from Unk to G after sequencing.

non-GBS-associated isolates possessed an LOS locus class (A, B, or C) capable of synthesizing a ganglioside mimic. Similarly, of the 21 isolates associated with enteritis, Godschalk et al. found that 62% possessed a class A, B, or C locus (9). It is not clear why the incidence of strains capable of producing ganglioside mimics from all sources is so high, although these findings seem to suggest that these ganglioside-mimicking LOS structures are advantageous to *C. jejuni* for colonization or

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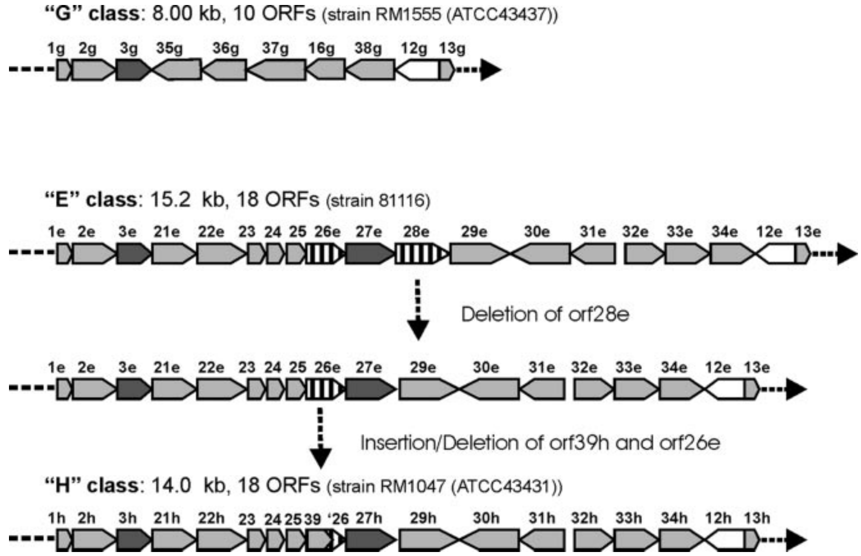

FIG. 2. Genetic organization of the LOS biosynthesis locus classes G and H and the proposed evolutionary events that resulted in generation of the class H locus from the class E locus. The GenBank accession numbers are AY436358 for strain RM1555 (ATCC 43437), AY800272 for RM1047 (ATCC 43431), and AF411225 for ATCC 43431 (4).

infection of various hosts. What is clear is that the production of ganglioside-mimicking LOS structures alone is not sufficient to elicit GBS; certain host factors and/or other bacterial factors are required.

Distribution of LOS classes within a group of Penner serotype isolates. Although there is strong evidence suggesting that the capsule rather than LOS is responsible for many Penner serotypes (12), it is possible that there remains a correlation between LOS genotype and Penner serotype. Indeed, all HS:19 and HS:41 strains examined share the same class A1 locus (see Tables 5 and 6). With only a limited number of strains possessing the same Penner type, we observed that Penner type and LOS class are not consistently associated. The HS:10 isolates (RM1556, RM1413, and RM1507) contained class A1 or A2, and the HS:2 isolates (RM1046, RM1188, RM1478, RM1480, and RM1862) contained class A1, B2, or C. Horizontal transfer of the LOS locus (or the capsular locus) provides a plausible mechanism for different LOS classes between strains with the same Penner serotype, as evidenced by the characterization of the class A1 LOS locus of GB11 (HS:2) (6). However, alternative mechanisms would be required to maintain the A1 LOS locus class in all the HS:19 and HS:41 strains examined in this study.

Identification of two new LOS locus classes. To verify novel LOS loci, we sequenced the XL PCR products from strains RM1555 (ATCC 43437) (GenBank accession number AY436358) and RM1047 (ATCC 43431) (GenBank accession number AY800272). The amplification pattern of RM1555 was inconsistent with the known LOS classes; it was positive for orf3e and *waaV* (orf12) products and negative for all other products. The sequence of the 5,537-bp region between *waaM* (Cj1134) and *waaV* (Cj1146c) establishes a seventh LOS locus class, G (Fig. 2). This locus contains four newly described open reading frames (ORFs) that encode putative glycosyltransferases based on BLASTX scores. A fifth potential ORF contains an intergenic homopolymeric G tract with 8 G bases (versus 9 G bases) that disrupts the reading frame after 336 nucleotides. By pairwise alignment, these 336 nucleotides show 93.5% identity to the first 322 nucleotides of the 888-nucleotide orf16d from RMLIO87 and 92.9% identity across the entire corresponding 888-nucleotide region. orf16f from RM1170 is similarly truncated, with 8 G bases in the homopolymeric G tract, and is 93.8% identical by pairwise alignment. The orf16df PCR product was not amplified from RM1555 due to significant sequence differences at the binding site of primer orf16df (data not shown). Interestingly, orf16d and orf16f are located adjacent to orf12d and orf12f in LIO87 and RM1170, respectively, but orf16g is separated from orf12g by orf38g, a pattern that presumably resulted from an insertion event.

The previous PCR results suggested that the LOS locus of RM1047 (ATCC 43431) differed from the class E locus but was similar to the presumptive class H locus. Indeed, the XL PCR product from RM1047 (ATCC 43431) was the same size as the class H LOS product (data not shown). Sequence analysis verified that this LOS locus represents a new class, termed class H, that is distinct from class E (Fig. 2). The orf28e gene is clearly missing from this locus, but the 3′ end of the orf26e gene is still present (Fig. 2). Furthermore, these changes appear to be the results of two different events. The absence of orf26e seems to be the result of an insertion-deletion event, with the 3′ end of orf25e still present and the 5′ region replaced by a putative butyryltransferase gene (orf39h). The second event appears to have been a deletion event that resulted in the complete removal of orf28e from RM1047, with minor changes to the 3′ end of orf27e and the 5′ end of orf29e. Considering these differences, we were able to reassign many of the isolates that were initially identified as class E to class H based on the PCR results for orf26e and orf28e (Tables 4 and 6). Furthermore, we identified hypervariable homopolymeric G tracts within orf23e (9 or 10 G bases) and orf25e (8 or 9 G bases). Therefore, all classes of LOS loci examined so far appear to have this common mechanism for creating LOS variability.

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Conclusions. This study reports a method to examine the diversity and classification of the LOS biosynthesis loci of *C. jejuni*. The PCR method identified the presence of genes from six LOS classes and was rapid, sensitive, and specific. The LOS structure of a particular isolate is determined not only by the genes of the locus but also by the presence of various mutations. Thus, strains sharing the same LOS class do not necessarily express the same LOS structure (7). Unfortunately, this method is unable to detect certain insertions, deletions, and point mutations that affect LOS structure. Nevertheless, we can infer LOS structures once an isolate's LOS class has been determined, particularly the presence or absence of ganglioside mimicry. Considering the potential role of *C. jejuni* ganglioside-mimicking structures in eliciting GBS or Miller Fisher syndrome, we believe that the LOS class of clinical strains provides valuable information and should be incorporated into epidemiological studies. Indeed, we found that all GBS-related strains and 64% of the other clinical and environmental isolates examined in this study belonged to an LOS class (LOS class A, B, or C) that allowed the synthesis of a sialylated LOS. The overrepresentation of HS:19 and HS:41 isolates likely biased the occurrence of LOS class A1 among GBS-associated strains. However, Godschalk et al. (9) similarly observed a significant association of LOS class A with GBS-associated isolates. Clearly, additional analysis with a more diverse group of GBS-associated strains is required to determine the significance of the LOS class.

Although not all LOS loci could be classified at this time, we demonstrated the ability to identify new loci, and we characterized the sequences of two of these, increasing the number of LOS classes to eight. For these new LOS classes, class G and class H, we observed evidence of genetic rearrangements (deletions or insertions) and homopolymeric G tracts that can lead to LOS structural variability. Specifically, the LOS synthesized by the LOS class H strain RM1047 could differ from those of other class H strains due to differences in the lengths of G tracts within biosynthesis genes. Also, LOS class H appears to be a derivative of class E, and therefore the LOS from these classes may share structural features. Additional analysis of *C. jejuni* LOS structures may allow potential structures to be inferred once an LOS class has been identified. Furthermore, the other unclassified LOS loci certainly substantiate the need for additional studies, and we envision the development of a microarray-based method that allows simultaneous analysis for distinguishing *C. jejuni* LOS classes.

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